Early apple fruit development under photoselective nets

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26 April 2024

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2   Alexandra Boini, Nicolò Casadio, Kushtrim Bresilla, Giulio Demetrio Perulli*, Luigi Manfrini, Luca

3   Corelli Grappadelli, Brunella Morandi.

4   Distal, University of Bologna. Viale Fanin, 46, 40127, Bologna, Italy.

5   *corresponding author: Giulio Demetrio Perulli

6

7   Abstract

8   The objective of this 2-year study (2017, 2019) was to evaluate the influence of photoselective

9   nets on apple fruit growth, focusing on the initial fruit growth stages of "*Pink Lady*". Trees were

10   subjected to four photoselective nets (Blue, Red, White and Yellow) and a standard black one

11   (serving as Control), resulting in 5 light environments (LE), all shading at 20%. From 20 to 90

12   DAFB, 32 fruit and extension shoots, for each LE, were measured for a total of 11 times during

13   the season. For each LE, fruit gauges were also installed to monitor fruit daily growth

14   parameters, from 50 to 90 DAFB. At harvest, all fruit from each light environment were

15   weighed and quality parameters were measured on 40 fruit per treatment. For each year,

16   correlations were made to test the influence of LEs on final fruit weight and quality, and the

17   impact that extension shoot growth had on fruit growth. In both years, white and control nets

18   led to the production of fruits with higher weight compared to the other treatments (blue, red

19   and yellow nets). In 2019, LEs did not appear to influence the relationship between shoot and

20   fruit growth, probably due to the higher crop load and the massive pruning carried out the

21   previous year. The higher vegetative outburst in this year might have unbalanced resources

22   towards the shoots, than towards the fruit. Results show how weather and orchard management

23   heavily influenced the trees responses. Yet, the consistent findings of final fruit weight, for both

24   years, indicate that different wavelengths influence fruit and shoot behaviour, even at early

25   phenological stages. Hence, growers searching for bigger fruit should refer to white and black

26   nets. Further studies approaching this technology can help improve apple production

27   management and knowledge of the use of photoselective nets.

28

29   KEYWORDS: apple, orchard, photoselective nets, fruit growth, fruit gauges, shoot growth,

30   yield.

31

32   1.   INTRODUCTION

33   To overcome the adverse effects of climate change, the use of protective nets, also called

34   anti-hail nets or shade nets, are becoming a common practice, since they protect orchards from

35   excessive solar radiation and hail damage. Such net installations are advisable in regions with

36   a high incidence of extreme weather events such as hail, often occurring in the Mediterranean

37   basin (Iglesias and Alegre, 2006; Özkaya et al., 2018).

38   Since protective nettings reduce solar radiation, they also modify the orchards'

39   microenvironment (Green et al. 2003). Netting also protects leaves from excessive light leading

40   to higher photosynthetic efficiency (Murata et al. 2006; Lopez et al. 2018). The most commonly

41   used types of net are black and white, whose physiological responses are widely known. The

42   addition of chromatic particles in the nets thread modifies light quality, thus its spectra, direction,

43   direction, diffusion, reflectance, transmittance and absorbance (Basile et al. 2008; Ganelevin,

44   2008; Shahak, 2008), plus its scattering, that can increase by 17-170% depending on the net

45   (Abdel-Ghany and Al-Helal, 2010). The use of photoselective nets has been studied in many

46   horticultural species (Stamps, 2009; Basile et al. 2012; Zoratti et al. 2015; Aoun and Manja,

47   2020; Gullo et al., 2021), but researchers are focusing on the impact of photoselective netting

48   in apple production systems.

49 These nets have proven to modify the orchard microenvironment with effects on relative

50 humidity, air and soil temperature (Iglesias and Alegre, 2006; Solomakhin and Blanke, 2010;

51 Arthurs et al. 2013; Kalcsits et al. 2017). They also directly affect sap flow rates, regardless of

52 the shading power (Boini et al. 2019). The orchard's geographic latitude location is,

53 nevertheless, the main variable that influences on the microenvironmental changes.

54

55 In apple, during the very early stages of fruit development, fruitlets are known to possess active

56 stomata (Blanke and Lenz, 1985), which contribute to active fruit transpiration. However, in

57 first half of the season, fruit growth is directly related to a high xylem functionality. This will

58 cause water backflows from fruit to stem when leaf transpiration losses are very high, followed

59 by strong rehydrations during late afternoon and night, when stomal conductance is reduced

60 (Lang, 1990; Morandi et al. 2012). In the second part of the season, fruit growth is almost

61 completely sustained by phloem flow, while the xylem vessels lose their functionality. This

62 marks the transition from an exponential growth model, to a linear one (Lakso et al. 1995). The

63 first, at the beginning of the season, is mainly characterized by cell division and an initial stage

64 of increasing growth rates. This phenological phase is known to highly influence final fruit

65 dimensions, as cell number can be a primary factor for fruit size (Lakso et al. 1989). The second

66 phase is characterized by cell expansion, therefore constant growth rates will last until harvest,

67 only with slight deaccelerations during the central parts of the day (Lang, 1990).

68

69   In light of the above information, it can be said that photoselective netting can be applied

70 to apple orchards, to improve certain aspects of its physiology. However, there appear to be no

71 studies focusing on the early stages of fruit growth. Considering the potential influence of these

72 nets on many physiological traits, assumptions can be made that there may be an impact on

73 fruitlet development and growth. Thus, the first objective of this study was to investigate on the

74   responses of fruit growth during cell division stage, under different photoselective nets, on a

75   daily and seasonal scale, to understand if light spectrum can modify early stages of fruit growth,

76   with consequences on final yield.

77   An important factor to keep into consideration during early fruit growth is the marked presence

78   of shoot elongation rates. Vegetative growth is known to negatively influence fruit

79   development, being a stronger sink, generally competing for photosynthetates (Grappadelli et

80   al. 1994). Given this unbalance in such a delicate phase that dictates final production, the second

81   objective of this study was to: understand if coloured nets can modify the competitive

82   relationship between vegetative and reproductive growth.

83   Results would allow to highlight or identify the possibility to optimize cell division stage, in

84   order to improve fruit quality and yield, in apple modern production. Insights of apple crop

85   responses to different light environments would also contribute to the pool of information

86   related to apple physiology under the effect of different light spectra.

87

88   2. MATERIALS AND METHODS

89   *2.1. Field and experimental set-up*. The trial took place at the experimental farm of the

90   University of Bologna, located in Cadriano (Bologna) (44°30′N; 10°36′E; 27 m elevation), in

91   an experimental apple orchard (*Rosy Glow*) grafted on M9, in seasons 2017 and 2019. Trees

92   were planted in 2015, spaced 3.3x1.0 m, with 10 rows of 50 trees each. Trees were trained as

93   the solaxe system in 2017, then modified to an open canopy system, between 2018 and 2019.

94   The orchard rows were North-South oriented, with concrete poles 3.8 meters high, sustaining

95   iron wires at three heights. Full bloom dates were 30 March 2017 and 29 March 2019. Around

96   20 days after full bloom (DAFB), in both years, four photoselective nets (red (R), blue (B),

97   white (W) and yellow (Y)) (ChromatiNet®,Polysack Industries, Negev, Israel) were placed

98 over sections of individual rows, as a mono-row cover, above 4 trees each (plus 2 serving as

99 guard trees). All four photoselective nets mesh measured 0.4x0.2 cm, had an English-turn

100 weaving system and had a shading percentage of around 20% (Corollaro, 2014). To avoid

101 interference between treatments, areas of around 11 meters in length and width were left

102 completely uncovered, around each plot (manufacture's suggestion). The rest of the orchard

103 was covered by a classic black anti-hail net (C) (Valente, Campodarsego, PD, Italy), as a roof

104 system, which served as control; this net measured 2.8x8 cm, had an English-turn weaving

105 system and shaded 20% (manufacturer statement). Also C had 4 monitored trees and these were

106 selected taking into account 11 meters distance from the coloured nets, plus another 11 meter

107 distance from the beginning of the forenamed net cover. The choice of such nets installation

108 comes from a limited amount of available space in the orchard (1500 m^2). The generated five

109 light environments (LEs) were repeated twice, thus, the trial was set to create a complete

110 randomized block design, where each LE had 8 monitored trees. The trial set up lasted until

111 harvest, in 2017; after harvest, coloured nets were taken off from the rows, while the control

112 net was rolled up, on top of the rows, a common practice in commercial orchards for Italy. In

113 2019, the trial set up lasted until the end of June (90 DAFB), after which, the whole orchard

114 was covered with the classic black hail net, thus all trees received the same quantity and quality

115 of light. It is assumed that LEs did not affect flower bud differentiation between seasons 2017

116 and 2019, since during year 2018 the orchard did not undergo any sort of trial (all trees were

117 covered with the same black control net).

118 *2.2. Light spectra assessment*. In winter 2017, a LI1800 spectroradiometer (LI-COR,

119 Lincoln, NE, USA) was used to assess the irradiance of the five LEs, on a cloudless day, at

120 midday and outside the orchard. The wavelength interval was between 400 and 1100 nm and

121 portions of each net were placed 20 cm over the sensor. Wm^{-2} outputs were converted to µmol

122 m^{-2} s^{-1}; transmittance was then calculated as the ratio between the external light input and the

123 light intensity under the net and expressed as a percentage.

124 *2.3. Weather parameters*. Meteorological conditions were measured using a weather

125 station (Wi-Net s.r.l. Cesena, Italy) installed outside the orchard. The following weather

126 parameters were recorded every 15 minutes: temperature, relative humidity, solar radiation,

127 wind speed and rain. Weather data was used to calculate environmental vapor pressure deficit

128 (VPD) and solar radiation (W m^{-2}) was converted to photosynthetically active radiation (PAR)

129 (μmol m^{-2} s^{-1}).

130 *2.4. Fruit growth*. From around 20 to 90 DAFB, in both years, fruit growth was

131 monitored both with a caliper and with automatic fruit gauges, for continuous measurements.

132 *2.4.1. Caliper measurements*. For each LE, 32 random fruit (4 $tree^{-1}$) were tagged and their

133 diameter was recorded with a digital caliper, connected to an external memory

134 (www.hkconsulting.it). Measurements were taken twice a week until the end of May, then once

135 a week in June, for a total of 11 times. At each measurement date, diameter data (D) from all

136 monitored fruit were converted to fresh weight (FW) using the following conversion equation:

137 $FW(g) = a * D(mm)^b$ (1)

138 where *a* and *b* were 0.0006 ($\pm$SE 0.00005) and 2.924 ($\pm$SE 0.0194). This equation was obtained

139 by regressing diameter and weight data of about 300 fruit picked from various *Pink Lady* apple

140 orchard (R^2 of the relationship was>0.99). Fruit absolute growth rate (AGR), expressed as g

141 day^{-1}, was then calculated using the following equation:

142 $AGR_{t1} = (FW_{t1} - FW_{t0})/(t_1 - t_0)$ (2)

143 *2.4.2. Continuous fruit growth measurements*. For each LE, 8 fruit were randomly selected and

144 their diameter was continuously measured using custom-built fruit gauges, as described by

145   Morandi et al. (2007). These consisted of a variable linear resistance transducer, supported by

146   a stainless-steel frame (Megatron Elektronik AG & Co., Munchen, Germany). A wireless data

147   logger system (Wi-Net s.r.l. Cesena, Italy) (Giorgetti et al. 2014), composed of five nodes,

148   received data from the fruit-gauges and communicated with a central coordinator. The network

149   coordinator acted as a gateway towards the internet, through a general packet radio service

150   (GPRS) modem.

151   Fruit were monitored at 15-minute intervals. At each monitoring interval, diameter data (D)

152   from all monitored fruit were converted to FW using the conversion equation (1). Thereafter,

153   fruit AGR was then calculated using the equation (2), thus by accumulating the hourly AGR,

154   fruit daily growth was then obtained, expressed in g day^{-1}.

155   In season 2017, technical issues did not allow to obtain data for the first 20 days after the

156   installation and made it impossible to collect data throughout the season from the B LE. For

157   homogeneity reasons, in both years, data are shown from approximately 50 to 90 DAFB. For

158   this continuous period of 40 days, the following fruit growth parameters were calculated for

159   each LE: hourly mean AGR (AGR$_{hour}$), mean gained weight, daily gained weight, mean

160   maximum AGR (AGR$_{max}$) and mean minimum AGR (AGR$_{min}$).

161   During these 40 days, four periods were selected: 50, 60, 70 and 90 DAFB. For each period, up

162   to 3 consecutive days were used as replicates (when meteorological conditions were the same),

163   to create daily patterns of fruit growth, in terms of AGR. For each period, the five previously

164   mentioned fruit growth parameters were further tested for each LE.

165   In general, when fruit gauges showed excessive noise or error values, they were excluded from

166   the data set.

167   *2.5. Extension shoot measurements*. From 20 to 90 DAFB of both years, 32 extension

168   shoots were randomly selected for each LE (4 tree^{-1}) and their extension was traced twice a

169   week until the end of May, then once a week during June. Measurements on shoots were

170   collected on the same dates as those for fruit' with the caliper. At each measurement date, length

171   data (L) from all monitored shoots allowed to calculate the absolute extension rate (AER), in

172   terms of mm day^{-1}, adapting equation (2) by substituting FW with L.

173   *2.6. Harvest and fruit quality*. At harvest (around 205 DAFB in 2017 and 207 DAFB in

174   2019) all fruit from each LE were calipered. Thereafter, the application of equation (1) allowed

175   to estimate the average fruit weight for each LE. Fruit quality parameters were measured on 40

176   representative fruit LE^{-1} (20 replication^{-1}). A slice of each fruit was used for soluble solid

177   content (SSC, °Brix), by measuring the refractometric index of the juice, with a HI 96811 digital

178   refractometer (Hanna, Woonsocket, RI), and for relative dry matter (RDM, %), calculated as

179   the percentage of dry weight relative to fresh weight, after drying samples in a forced air oven

180   at 60°C.

181   *2.7. Correlations study*. For each year, the following correlations were obtained.

182   i)   Average fruit weight and quality parameters (SSC and RDM) were related to fruit

183   growth parameters obtained from fruit gauges, during the continuous 40-day period.

184   ii)   Fruit growth parameters obtained from fruit gauges, during the continuous 40-day

185   period, were related to crop load.

186   iii)   Fruit AGR for each LE, obtained with caliper measurements, were related to the

187   extension shoot AER.

188

189   *2.8. Statistical analysis*.

190   *2.8.1. Light spectra assessment*. A one-way ANOVA was used for separating the averages

191   among blue light band (400–500 nm), green (500-600 nm), yellow-red band (600-800 nm),

192   PAR (400–700 nm) and infrared band (IR) (> 700 nm).

193 *2.8.2. Weather.* A one-way ANOVA was applied for evaluating differences every 10 days for

194 environmental VPD and mean PAR between 9 and 18 h, between the two seasons.

195 *2.8.3. Continuous fruit growth measurements.* A one-way ANOVA was used for testing

196 differences between LEs for fruit growth parameters, for each year. A two-way ANOVA was

197 used for testing differences between LEs fruit AGR_{hour}, during daily time, for each year.

198 *2.8.4 Extension shoot measurements.* A one-way ANOVA was used for testing differences

199 among LEs.

200 *2.8.5. Harvest parameters.* Crop load was analyzed between treatments, revealing no

201 significant difference for each year, thus a one-way ANOVA could be used to test differences

202 for average fruit weight.

203 *2.8.6. Correlations study.* Correlations i) and ii) were analyzed and the correlation coefficients,

204 slopes and p-values reported. Regarding correlation iii), each LE relation was analyzed,

205 reporting the correlation coefficients and p-values. A repeated measures analysis followed, after

206 which slopes were tested for the effects of LE, AER, time and AER*time.

207 SNK's test (Zar, 1984) was then applied to rank the averages, in all the reported analyses,

208 considering significant P<0.05.

209

210 3. RESULTS

211 *3.1. Light spectra assessment.* Spectral distribution of light transmitted under tested nets

212 (Fig. 1) showed transmittance patterns typical of their different colour. In the blue (400-500

213 nm) (Fig. 2A) and green (500-600) areas (Fig. 2B) every treatment was different, W having

214 highest transmission and R the lowest, leaving the other LEs alternating as intermediates. In the

215 yellow-red area (600-800) (Fig. 2C) Y was highest and B lowest, while in in the infra-red region

216   (< 700 nm) (Fig. 2D) values were higher for C and lower for B and R. PAR (400-700 nm) (Fig.

217   2E) was more transmitted under the W net (around 75%) and less under the R LE (around 65%).
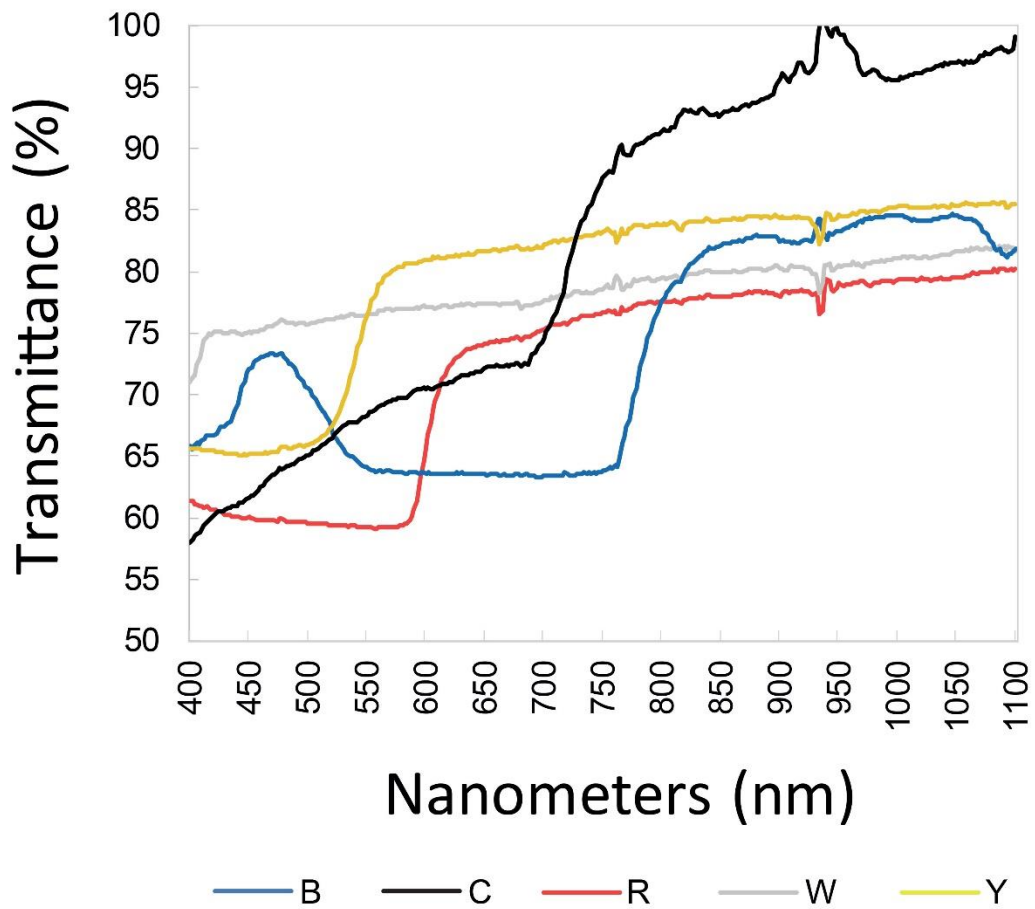
218

219   *Figure 1. Spectral light transmittance under nets, ranging from 400-1100 nm.*
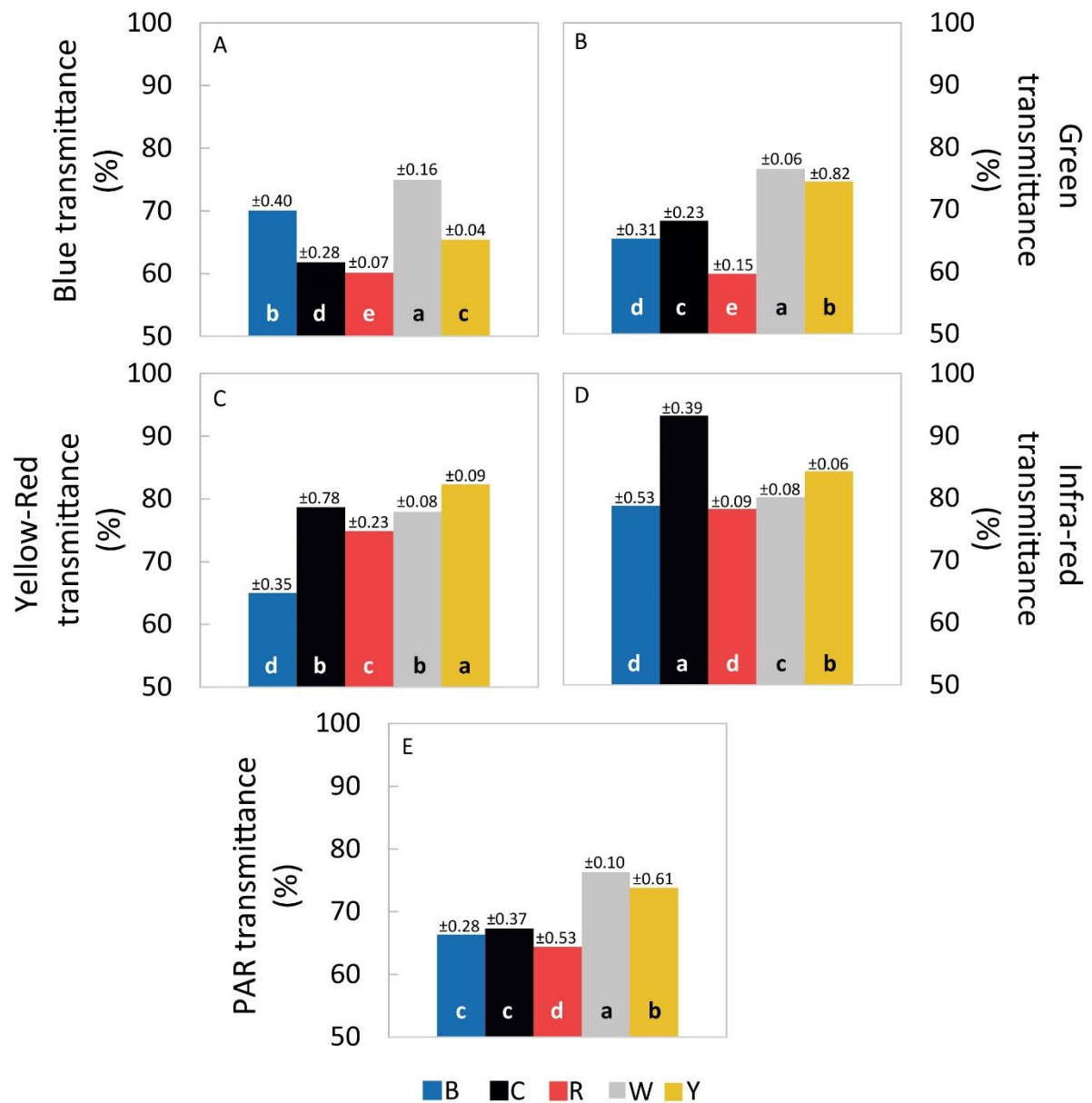
Figure 2. Transmittance mean values for blue (A), green (B), yellow-red (C), infra-red (D) and PAR (E) wavebands, under each LE. Different letters indicate significant difference at P<0.05. Numbers on top of each column are standard error values.

3.2. Weather parameters. Between the two years, 2017 showed generally higher PAR and VPD values, especially from 40 DAFB on.

In 2017, from 23 to 61 DAFB, the maximum solar radiation was stable around 2000 μmol m^{-2} s^{-1}, moreover, it reached peaks of over 2300 μmol m^{-2} s^{-1}, while the average PAR, measured

229   between 9 and 18 h, was about 1150 µmol m^{-2} s^{-1}, but exceeded 1300 µmol m^{-2} s^{-1}, 11 times

230   during the season. The period going from 62 to 91 DAFB was characterized by slightly lower

231   peaks of maximum solar intensity (peaks above 2000 µmol m^{-2} s^{-1} only 10 times) and the mean

232   PAR was about 1280 µmol m^{-2} s^{-1}, reaching peaks above 1300 µmol m^{-2} s^{-1} 22 times (Fig. 3A)

233   during the season.

234   In 2019, the weather conditions were considerably different. From 20 to 63 DAFB, the solar

235   radiation was frequently below 800 µmol m^{-2} s^{-1}, reaching peaks over 1200 µmol m^{-2} s^{-1} just 4

236   times and a single one of over 2000 µmol m^{-2} s^{-1}. The second period, from 64 to 91 DAFB was

237   characterized by higher solar intensity peaks (peaks above 1300 µmol m^{-2} s^{-1} almost every day)

238   and the average PAR was about 1000 µmol m^{-2} s^{-1} (Fig. 3A).

239   Also, VPD was different between the two years, especially from 40 DAFB to 60 DAFB. In

240   2017 the average of this period was 0.75 kPa, while in 2019 the medium was 0.35 kPa. The

241   average recorded value in 2017, from 20 to 90 DAFB, was 0.88 kPa and in the same period of

242   2019, VPD reaches the same value (0.87 kPa) (Fig. 3B).

243
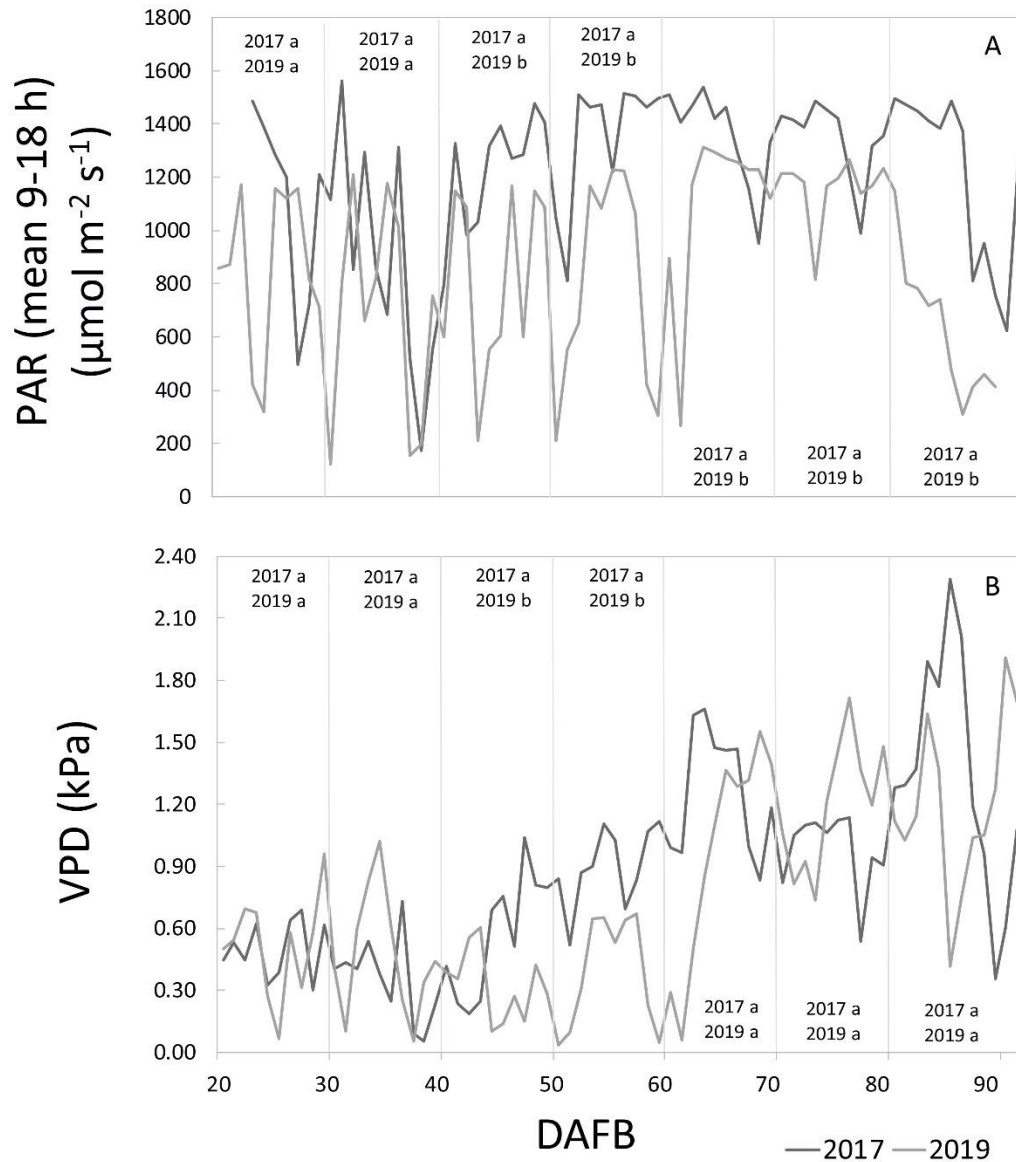
Figure 3. Comparison of mean PAR between 9-18h, for 2017 and 2019 referred to DAFB (A). Comparison of VPD in the two years, referred to the DAFB (B). The presence of different letters indicates significant difference at P<0.05.

3.3. Fruit growth. Daily fruit growth (Fig. 4) showed significant differences. In 2017, at 50 DAFB, daily growth and most fruit growth parameters were similar for each LE (Fig. 4A, Table 1), with periods of rapid growth recorded in the late afternoon and at night, followed by periods of reduced or even negative growth during the central part of the day (Fig.4A). Only Y had the highest mean gained weight (0.187 g day^{-1}). At 61 DAFB, there was a significant

253   difference between LEs, more precisely, Y tended to have higher values (Fig. 4C, Table 1),

254   possibly because of a higher AGR_{max} (Table 1).

255   With fruit development, shrinkage was replaced by a steady state for some LEs (at 77 DAFB

256   and 91 DAFB) (Fig. 4E-G). At 77 DAFB, significant differences can again be found, and the R

257   treatment presents a significant rehydration, from 17:00 to about 24:00 h and the mean gained

258   weight was significantly higher (0.63 g day^{-1}). On this date, R and W had a midday shrinkage

259   phase, compared to C and Y where there was no shrinkage, however with no differences during

260   most of the day. Yet, Y net also reached high mean gained weight values(0.69 g day^{-1}), like R,

261   a possible result given by the lack of shrinkage. At 91 DAFB the situation was similar among

262   all treatments, although W growth exceeded 1.20 g day^{-1} (Table 1), unlike B (Table 1) which

263   had lower values for AGR_{hour} and fruit daily growth.

264
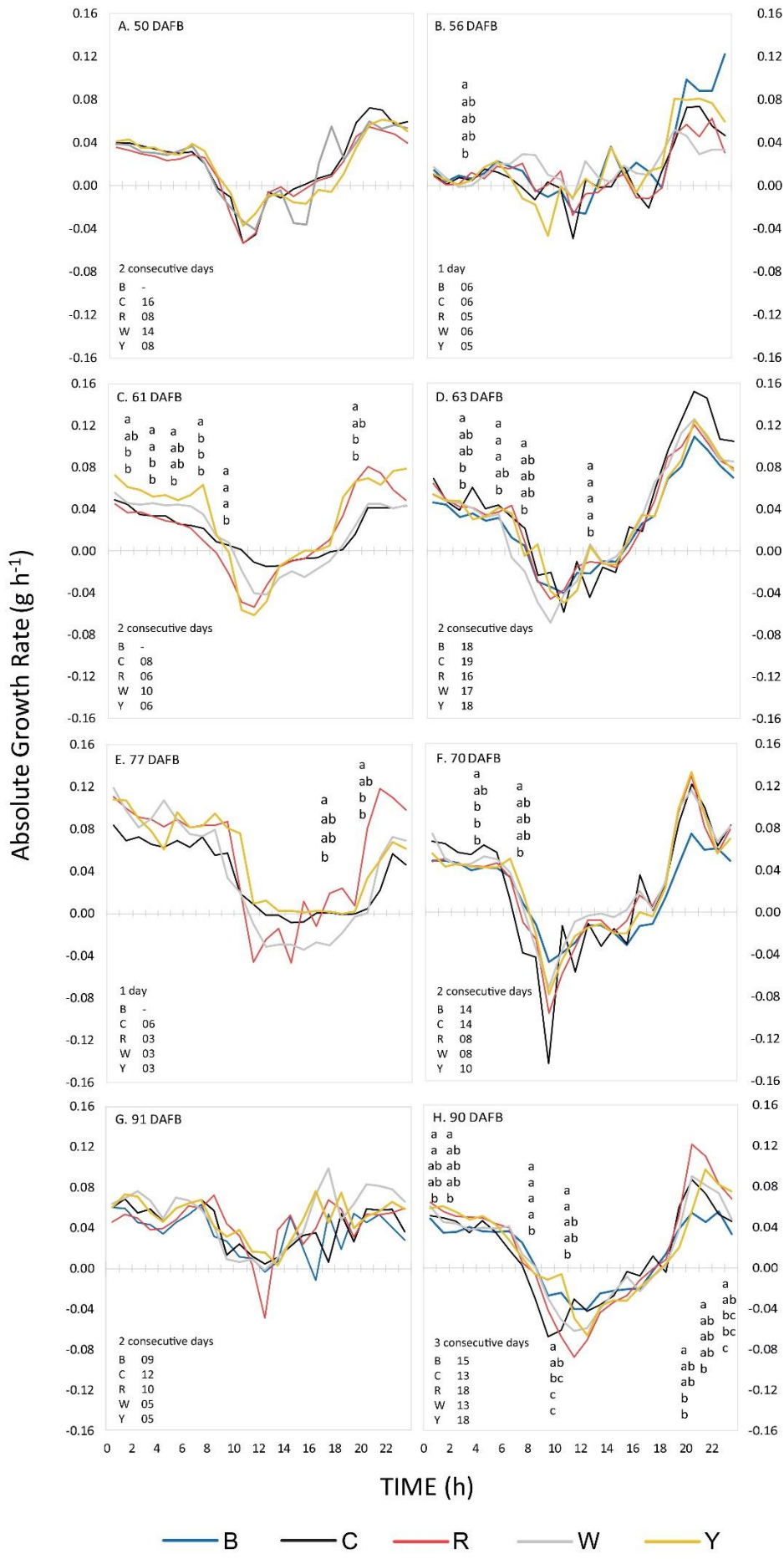
Figure 4.

Fruit daily AGR, for season 2017 (on the left) and season 2019 (on the right), under B (blue lines), C (black lines), R (red lines), W (light grey lines) and Y (yellow lines) LEs, at four significant periods.

For each period, the number of days used for analyses and the number of used fruit gauges, for each LE, are reported in the bottom left corner.

Different letters indicate significant difference at P<0.05. No letters indicate no significant differences.

In 2019, at 56 DAFB (Fig. 4B) the trend was not characterized by marked contractions in the central hours of the day, however growth rates were very low, without differences between LEs. Nevertheless, table 1 shows higher fruit mean gained weigh under the W (0.150 g day^{-1}) net and lower ones under the C (0.044 g day^{-1}).

A week later (Fig. 4D), daily growth increased, showing peaks of rehydration during the night and early morning. During these hours, there were significant differences among LEs, whereas in the middle of the day all treatments seemed to react in the same way. W tended to have the lowest values from 3:00 to 12:00 h. Fruit growth parameters at 63 DAFB (Fig. 4D, Table 1), were significantly different, C having higher values especially when compared to B and W.

At 70 DAFB (Fig. 4F), midday shrinkage was still present, with no marked differences among LEs. Yet, C presents the lowest value, in terms of mean gained weight (0.128 g day^{-1}) (Table 1), nearly half compared to W (0.224 g day^{-1}).

In the last period, at 90 DAFB (Fig. 4H), in the first hours of the day, Y fruit grew more than the other LEs, then, before midday C and R tended to shrink more, along with W (values below -0.05 g hour^{-1}) ones. B fruit had intermediate behaviour firstly, whilst became the lowest by the end of the day, in terms of g hour^{-1}. Among fruit parameters, differences were present for mean gained weight, where Y was highest (0.165 g day^{-1}) and C was lowest (0.07 g day^{-1}).

Table 1. Average fruit growth parameters (horizontally listed) values, followed by SE, for each light environment (vertically listed) in 2017 and 2019. For each date, different letters among rows indicate significant difference at P<0.05. ns indicates no significance.

Year	DAFB	LE	AGR$_{hour}$			Mean gained weight			Total gained weight			AGR$_{max}$			AGR$_{min}$		
			(g h^{-1})	SE		(g day^{-1})	SE		(g day^{-1})	SE		(g)	SE		(g)	SE	
2017	50	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		C	0.021	0.007	ns	0.165	0.022	ab	0.447	0.044	ns	0.076	0.006	ns	-0.067	0.013	ns
		R	0.015	0.006	ns	0.135	0.017	c	0.350	0.014	ns	0.056	0.004	ns	-0.060	0.017	ns
		W	0.018	0.006	ns	0.152	0.019	bc	0.395	0.056	ns	0.078	0.007	ns	-0.072	0.013	ns
		Y	0.018	0.006	ns	0.187	0.019	a	0.412	0.035	ns	0.067	0.005	ns	-0.058	0.010	ns

Year	Num	Type														
	61	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		C	0.017	0.004	b	0.189	0.016	b	0.356	0.081	ns	0.067	0.012	b	-0.027	0.007	ns
		R	0.018	0.008	b	0.120	0.022	c	0.378	0.061	ns	0.088	0.007	ab	-0.059	0.008	ns
		W	0.016	0.007	b	0.203	0.018	b	0.335	0.111	ns	0.089	0.014	ab	-0.073	0.015	ns
		Y	0.029	0.009	a	0.293	0.030	a	0.629	0.055	ns	0.119	0.009	a	-0.075	0.026	ns
	77	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		C	0.034	0.007	ns	0.489	0.042	b	0.731	0.151	ns	0.099	0.018	ns	-0.008	0.004	ns
		R	0.052	0.011	ns	0.630	0.057	a	1.133	0.186	ns	0.147	0.012	ns	-0.073	0.032	ns
		W	0.035	0.011	ns	0.483	0.037	b	0.659	0.328	ns	0.130	0.032	ns	-0.039	0.026	ns
		Y	0.050	0.008	ns	0.693	0.063	a	1.093	0.120	ns	0.122	0.008	ns	-0.003	0.002	ns
	91	B	0.035	0.004	b	0.428	0.048	b	0.840	0.178	ns	0.129	0.028	ns	-0.057	0.047	ns
		C	0.041	0.004	ab	0.510	0.054	ab	0.979	0.162	ns	0.120	0.025	ns	-0.038	0.019	ns
		R	0.043	0.005	ab	0.488	0.058	ab	0.990	0.143	ns	0.135	0.021	ns	-0.091	0.046	ns
		W	0.052	0.006	a	0.582	0.070	a	1.275	0.121	ns	0.146	0.026	ns	0.011	0.020	ns
		Y	0.050	0.004	ab	0.569	0.063	ab	1.108	0.219	ns	0.126	0.013	ns	-0.005	0.013	ns
2019	56	B	0.023	0.008	ns	0.110	0.028	ab	0.542	0.141	ns	0.093	0.012	ns	-0.039	0.005	ns
		C	0.012	0.006	ns	0.044	0.016	c	0.284	0.095	ns	0.090	0.017	ns	-0.081	0.029	ns
		R	0.012	0.005	ns	0.071	0.014	bc	0.274	0.058	ns	0.102	0.013	ns	-0.059	0.013	ns
		W	0.018	0.003	ns	0.150	0.025	a	0.413	0.168	ns	0.096	0.028	ns	-0.043	0.017	ns
		Y	0.018	0.007	ns	0.069	0.025	bc	0.425	0.071	ns	0.105	0.021	ns	-0.060	0.031	ns
	63	B	0.027	0.009	b	0.167	0.033	c	0.601	0.080	b	0.160	0.014	ns	-0.069	0.013	ns
		C	0.041	0.012	a	0.286	0.053	a	0.983	0.109	a	0.213	0.029	ns	-0.100	0.016	ns
		R	0.034	0.010	ab	0.243	0.042	b	0.785	0.058	ab	0.161	0.012	ns	-0.081	0.013	ns
		W	0.031	0.011	b	0.194	0.042	c	0.741	0.055	ab	0.165	0.016	ns	-0.102	0.011	ns
		Y	0.033	0.009	ab	0.237	0.039	b	0.747	0.050	ab	0.163	0.015	ns	-0.083	0.014	ns
	70	B	0.016	0.007	ns	0.149	0.019	bc	0.338	0.136	ns	0.107	0.014	ns	-0.093	0.017	ns
		C	0.021	0.012	ns	0.128	0.031	c	0.446	0.116	ns	0.185	0.034	ns	-0.149	0.021	ns
		R	0.022	0.010	ns	0.146	0.029	bc	0.489	0.101	ns	0.163	0.021	ns	-0.113	0.033	ns
		W	0.030	0.009	ns	0.224	0.032	a	0.638	0.074	ns	0.162	0.019	ns	-0.088	0.025	ns
		Y	0.024	0.010	ns	0.195	0.023	ab	0.450	0.076	ns	0.119	0.011	ns	-0.091	0.015	ns
	90	B	0.013	0.007	ns	0.126	0.017	b	0.259	0.046	ns	0.084	0.005	b	-0.061	0.007	a
		C	0.013	0.009	ns	0.077	0.023	c	0.248	0.071	ns	0.134	0.008	a	-0.149	0.016	c
		R	0.017	0.012	ns	0.107	0.030	bc	0.342	0.042	ns	0.142	0.011	a	-0.107	0.009	b
		W	0.014	0.009	ns	0.110	0.023	bc	0.287	0.051	ns	0.107	0.011	ab	-0.076	0.006	ab
		Y	0.018	0.009	ns	0.165	0.022	a	0.363	0.049	ns	0.115	0.010	ab	-0.082	0.010	ab

319

3.4. Extension shoot measurement. The average behaviour of shoots during the monitored periods was different between the two years. In 2017, extension shoot growth did not show significant differences (Table 2) and all the values were between 0.21 mm day^{-1} (B) and 0.28 mm day^{-1} (R).

324 In 2019, there were significant differences between the LEs (Table 2). The highest values were

325 obtained under B (0.86 mm day^{-1}) and R (0.88 mm day^{-1}), different from W (0.65 mm day^{-1})

326 and even more different from C (0.54 mm day^{-1}) and Y (0.50 mm day^{-1}) (Table 2).

327

328 *Table 2. Values of shoot extension growth (mm day^{-1}) for 2017 and 2019, followed by SE. Different letters*

329 *indicate significant difference at P<0.05, while ns indicates no significance.*

	25-91 DAFB (2017)			26-90 DAFB (2019)		
LE	mm day^{-1}	SE		mm day^{-1}	SE	
B	0.20	0.019	ns	0.87	0.038	a
C	0.25	0.021	ns	0.54	0.036	c
R	0.26	0.019	ns	0.88	0.040	a
W	0.25	0.021	ns	0.64	0.035	b
Y	0.25	0.020	ns	0.51	0.033	c

330

331

332 *3.5. Harvest, fruit weight and quality.* In 2017, the total load did not present differences

333 and all the values were between 50 and 80 fruit tree^{-1} (Fig. 5A). Fruit weight values ranged

334 from 182 to 202 g and all LEs were significantly different from each other (Fig. 5B). The highest

335 value was reached with W (202.37 g), the lowest with B (182.75 g), while the other LEs were

336 close to 190 g.

337 B, R and Y fruit showed the lowest SSC, with values around 13.5 °Brix, while C and W, reached

338 14.5 and 14 °Brix (Fig. 6A), respectively. Instead, RDM did not present significant differences

339 with all values above or close to 17% (Fig. 6B).

340 In 2019, the situation changed. The total load went from 130 to 180 fruit tree^{-1} (Fig 5C), and

341 this affected the total marketable yield, in fact, the highest average fruit weight value was

342 163.73 g, for fruit grown under C net, while the lowest reached around 150 g, under both R and

343 Y nets (Fig. 5D).

344 Regarding SSC and RDM, the first were significantly higher in B and C with values close to

345 12.5 °Brix, while the other treatment resulted lower, less than 12 °Brix (Figure 6C). RDM (Fig.

346   6D) showed significant differences among LEs: B showed higher values (0.25%) than C, W

347   and Y which were similar, while all treatments were higher than R (0.23 %).
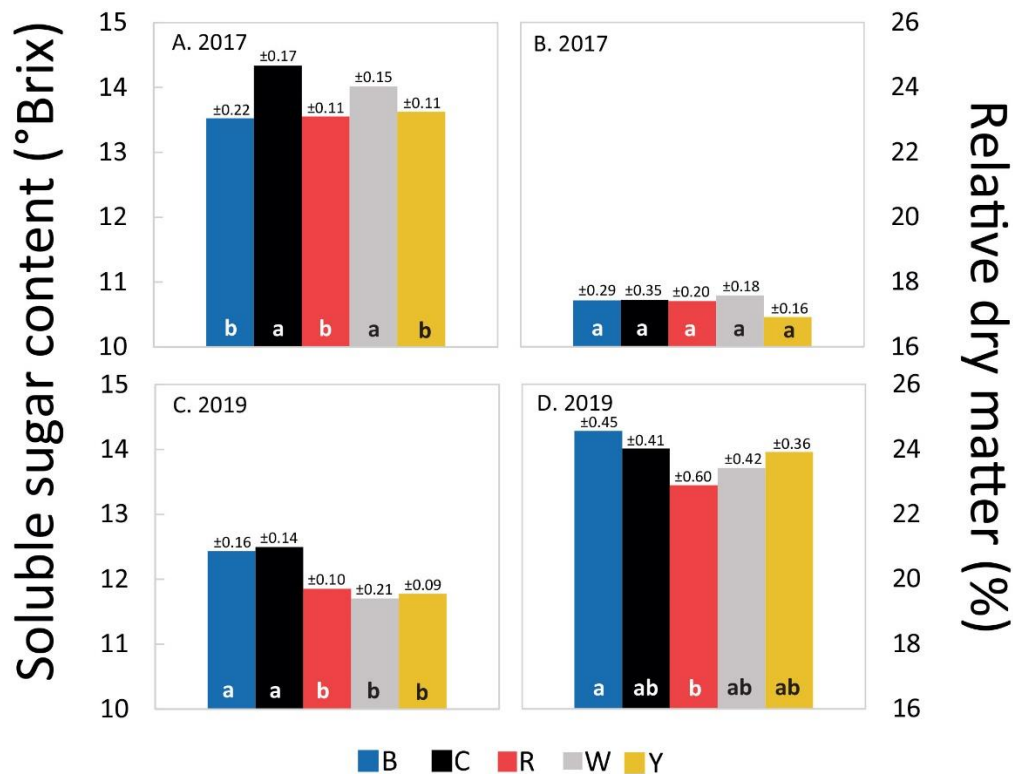
348
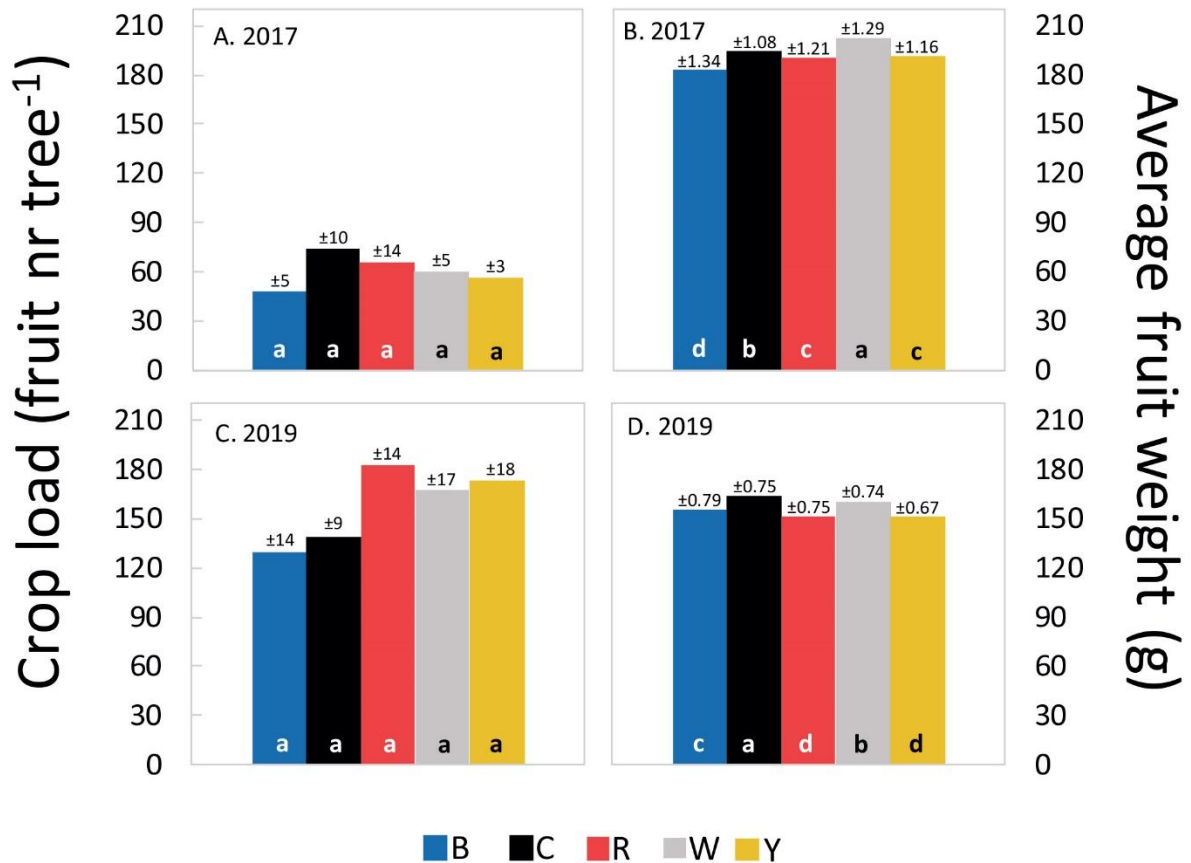
349

350   *Figure 5. Crop load and average fruit weight of blue (B), control (C), red (R), white (W) and yellow (Y) LEs. Plots*

351   *A and B are referred to 2017, while C and D are referred to 2019. Different letters, among columns, indicate*

352   *significant difference at P<0.05. Numbers on top of each column are standard error values.*

353

354

Figure 6. Soluble sugar content and relative dry matter of blue (B), control (C), red (R), white (W) and yellow (Y)

LEs. Plots A and B are referred to 2017, while C and D are referred to 2019. Different letters, among columns,

indicate significant difference at P<0.05. Numbers on top of each column are standard error values.

3.6. Correlations study. Fruit quality parameters (fruit weight, SSC and RDM), related

to fruit growth parameters (AGR_{hour}, mean and total gained weight, AGR_{max} and AGR_{min}), did

not show significant relationships in both years (results not shown).

Fruit growth parameters, cited above, related to the crop load in the two seasons were significant

only in 2019, when a significant positive correlation was found for AGR (hourly mean) (r 0.90,

p 0.04) and total gained weight (r 0.91, p 0.03).

A significant negative relationship between AGR and AER was also found (Fig. 7A, B). In

2017, r values were always above 0.70. The relation between AGR and AER is strictly different

in 2019. The r values above 0.70 in C, R and Y highlight the non-randomness of data while this

was not true for B and W that had values of 0.52 (B) and 0.62 (W). Nevertheless, p values

highlight the significance of the relationships and all treatments had p values below 0.05 except

B and R with a p of 0.15 and 0.075, respectively.
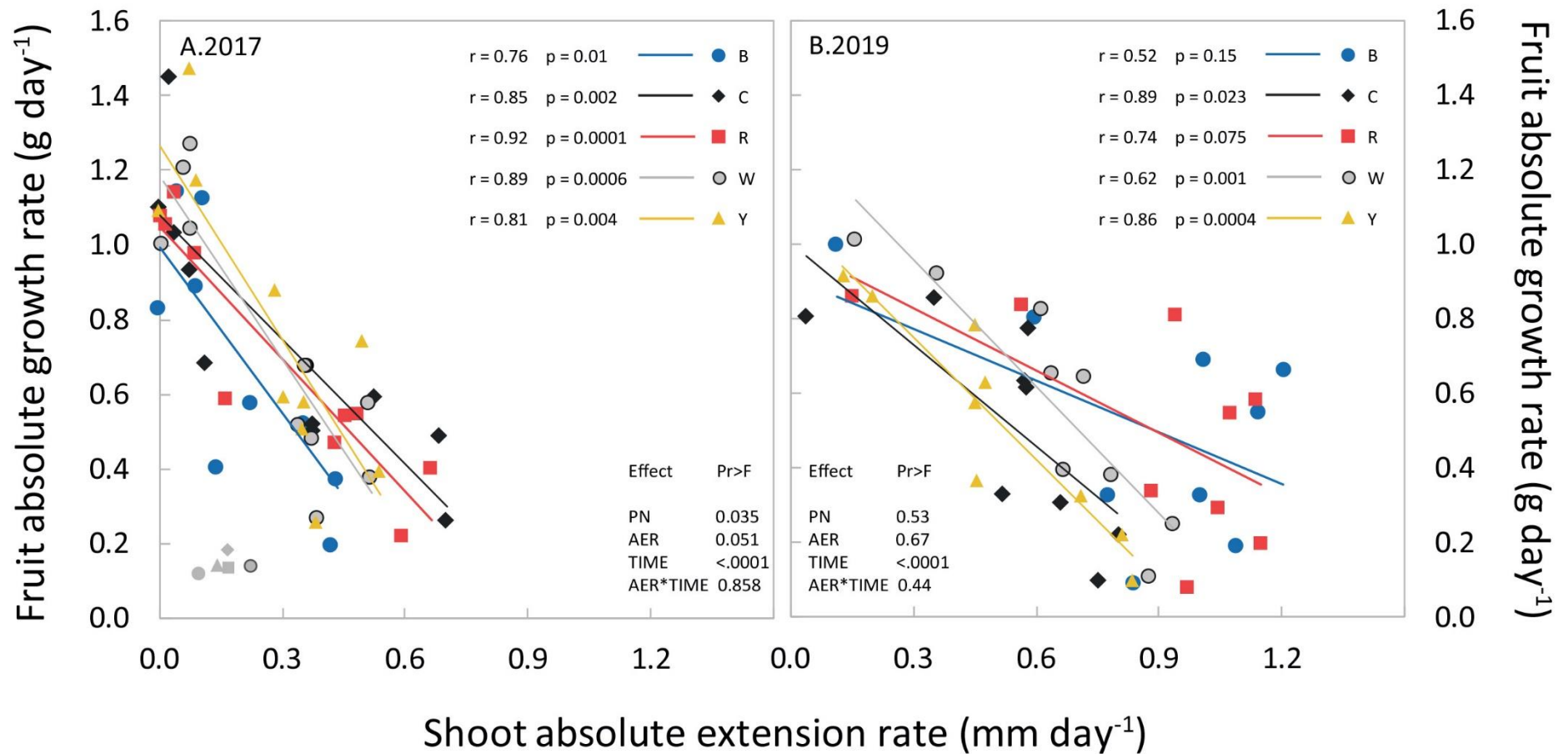
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371

372   *Figure 7. Relationships between shoot absolute extension rate and fruit absolute growth rate obtained with caliper measurements, of each LE, for 2017 (A) and 2019 (B).*

373   *Each symbol represents a date of measurement, thus the mean of 32 extension shoots and 32 fruit. The effects of LE, AER, TIME and AER*TIME are shown (Pr>F). For*

374   *each relationship, r and p values are reported. The grey cluster of symbols in the bottom left corner (A) represents a group of outliers.*

4. DISCUSSION

One of the objectives of this work was to detect the possibility that coloured nets could modify the early stages of fruit growth, with consequences on final fruit weight. Regardless of the very different crop loads between the two years (Fig. 5A, 5C), average fruit weight seemed to follow a similar behaviour. Higher values were reached for fruit under the W and C LEs (Fig. 5B, 5D), whereas the other LEs were always lower. SSC and RDM results in the two years (Fig. 6), did not report similar trends, except for °Brix, with higher values for C fruit and lower ones for R fruit (Fig. 6A, 6C).

Another objective of this work was to evaluate how photoselective nets can influence fruit growth, during cell division stage. Since technical issues occurred, the discussed data covers the final period of cytokinesis.

In 2017, fruit daily growth at 50 (Fig 4A) and 61 DAFB (Fig 4C) was characterized by shrinkage during the central hours of the day, for all LEs. Shrinkage was more or less pronounced, with no significant differences among treatments (Fig. 4C). The same periods in 2019 showed a similar behaviour. At 56 DAFB (Fig. 4B) no significant differences were present, indicating that LEs were possibly not influencing fruitlet growth enough. Significant differences among LEs were present at 63 DAFB (Fig. 4D), where C fruit tended to be higher for most of the first part of the day. Shrinkage was present, however no differences occurred during the central part of the day.

At 77 DAFB and 91 DAFB a change in fruit growth behaviour took place. In 2017, C and Y LEs did not shrink during the central hours of the day, unlike R and W (Fig. 4E), suggesting that their fruit xylem might be rather dysfunctional as it usually occurs at this time of apple fruit development (Lang, 1990). These differences in the fruit daily growth pattern suggest a transitioning phase for these specific LEs. Unluckily, no significant differences among the four LEs allowed to validate this statement. The lack of significance is probably caused by the very

400   low number of replicates that were used for this specific period (Fig. 4E). At 91 DAFB (Fig.

401   4G) all LEs do not present shrinkage.

402   In the second phases of 2019, from 70 to 90 DAFB (Fig. 4 F-H), all LEs had a marked shrinkage

403   during the central hours of the day, showing a still active xylem. Compared to 2017, mean and

404   total gained weight values are much lower (Table 1). This pronounced difference is probably

405   caused by the weather, in fact spring 2019 (precisely, until 60 DAFB) had lower VPD values,

406   with low temperature and consequently delayed fruit development, causing slow growth and

407   delaying xylem dysfunctionality (Lang, 1990). Another probable cause of delayed xylem

408   closure was the lower incidence of PAR in 2019, which showed lower values compared to 2017.

409   The environmental differences between the two years thus played a major role in dictating fruit

410   growth, as stated by Stampar et al. (2002), regardless of LEs.

411   A final remark has to be dedicated to W net daily growth, in 2017. Although this treatment had

412   the highest fruit weight at harvest (Fig. 5B), it did not appear to have higher growth rates (Fig.

413   4A-C-E-G, Table 1) during the initial growth stages. It is believed that the fruit gauges of this

414   specific light environment were installed on fruit that were not representative (i.e. with low

415   growth rates). Moreover, in some cases, appropriate data was not available, leaving down to 3

416   sensors LE^{-1}. Such a low number of replications in some cases may not be enough to generate

417   significant differences (Fig. 4E), caused by a less dispersion of the statistical error.

418   Fruit growth during the first part of the season can be modified with the use of photoselective

419   nets, however, differences occurring at harvest (average fruit weight) may not reflect spring

420   and early summer growth. As literature states (Pretorius et al. 2004; Okello et al. 2015), cell

421   expansion phase is important for determining final fruit weight, since this phenological stage

422   can also be influenced by external factors, such as weather, water availability and light. It has

423   to be underlined that weather and orchard management play a major role in dictating the

424   development of fruit. Less warm and sunny springs, plus vigorous shoot outburst, slow down

425   reproductive growth (Wünsche and Lakso, 2000), not to mention the effects of crop load (Naor

426   et al. 1997), this latter factor being significant for fruit growth parameters in 2019.

427   The last objective to analyse, was to understand if coloured nets can modify the

428   competition between vegetative and reproductive growth.

429   Lakso and Goffinet (2013) noted how, about 3-4 weeks after bloom, the tree urges vegetative

430   growth, to the detriment of the productive yield (Grappadelli et al. 1994). In Table 2, year 2017

431   showed no significant differences, regardless of LEs, known to impact on vegetative growth

432   (Shahak et al. 2004a, 2004b; Mupambi et al. 2018). In 2019, the situation was completely

433   different, and LEs seemed to influence vegetative growth. A possible cause, of such pronounced

434   growth, could be the heavy pruning, which took place in the former winter. Thereafter, it is

435   possible that the higher crop load (Fig. 5C), along with higher vegetative growth, contributed

436   to lower fruit weight at harvest (Naor et al. 2008; Radivojevic et al. 2012). In, 2019, B and R

437   LEs showed the highest vegetative extension (Table 2), thus shoots were favoured, i.e. they

438   received more photosynthates, with the results of lighter fruit (Fig. 5B-D). On the contrary, in

439   C and Y the translocation towards the fruit was favoured, as confirmed by the average higher

440   fruit weight values (Fig. 5B-D). This is important, because with the different LEs used, under

441   different climatic conditions, different results are expressed that could be interesting to improve

442   vegetative growth, instead of reproductive growth.

443   The correlations between AGR and AER in 2017 (Fig. 7A) showed differences for all LEs with

444   r values above 0.7 and p-values below 0.05, which highlight the competition between shoot and

445   fruit (Wunsche and Lakso, 2000; Grappadelli et al. 1994). In 2019 (Fig. 7B), C, Y and W have

446   significant relationships between AGR and AER. This underlines that B compared to the other

447   LEs did not impact in the same way on the competition between the two structures, leading to

448   contradicting results. The slope analysis for 2017 (Fig 7A), highlights significant effects of LEs

449   and only slightly of AER. In 2019 (Fig. 7B), neither LEs nor AER appeared to influence fruit

450   AGR. This difference between the two years is probably due to the different crop load in these

451   seasons, in fact, there was a significant relationship between crop load, AGR_{hourly} and total

452   gained weight, for season 2019 (r values of 0.90 and 0.91). In 2017, there was a lower number

453   of heavier fruits, while in 2019 there was a higher number of fruits with a lower weight. This

454   means that in 2017 the competition for photosyntates between fruit and shoots was lower,

455   pushing more towards the fruit; instead, in 2019 the influence of shoots was markedly higher,

456   caused by the heavier pruning carried out in 2018 which led to a higher number of sprouts.

457   In both years, among the coloured nets, higher fruit weights were obtained under C and

458   W nets. In the first case, this could be a consequence of the different types of net installation.

459   If coloured nets were installed on individual rows, C net was left open as to cover the rows as

460   the traditional roof system. Thus, this last cover potentially allowed higher incoming radiation

461   to reach the trees. Higher growth values would be due to a higher amount of total light filtering

462   below the C treatment. Therefore, light quantity was more effective than light quality.

463   In the second case, the W net alternated significantly higher, to intermediate, to lower values of

464   fruit growth, during 2017 and 2019, for all the observed parameters (Fig. 4, Table 1).

465   Surprisingly, it harvested bigger fruit, compared to the other LEs (Fig. 5B,D). This is probably

466   caused by the higher transmittance in the blue light regions (Fig. 2A) and PAR (Fig. 2E)

467   (Bastías, 2011; Kong et al. 2012), that may have modified leaf anatomy, enlarging the stomata

468   (Kong et al. 2012) and consequently improving the photosynthetic performance. Fruit growth

469   parameters measured with fruit gauges did not allow to detect such information, probably due

470   to the low number of replicates which was not enough and/or the selected apples were not

471   representative of the LE.

472   The Y net was able to favour fruit growth, in 2017, possibly thanks to the higher light scattering

473   properties of this net (Shahak et al. 2016), explaining the higher AGRs (Table 1). Another

474   possible reason could be the high transmittance in the PAR region (400-700 nm) (Fig. 2E), in

475   fact, this factor added to the high scattering proprieties, may have enriched the light

476   microenvironment (Hemming et al. 2016) enough to justify higher growth rates, although not

477   for the whole season (Fig. 4, Table 1). Consequently, harvest results did not reach high values,

478   as the W ones (Fig. 5B). In 2019, Y net showed lower values for extension shoot and fruit

479   growth rates (Table 1), compared to 2017. Several studies (Dougher and Bugbee, 2001; Kim et

480   al. 2004) give diverse opinions about the impact of this specific wavelength, along with the

481   transmittance of green light. It has been discovered, however, that yellow wavelengths

482   inactivate cryptochromes and increasing green light has been shown to reverse the blue-light-

483   dependent stomata opening response, thus reducing stomatal conductance in many herbaceous

484   crops (Frechilla et al. 2000; Talbott et al. 2002; Kim et al. 2004), with a dose-dependent

485   behaviour. The spectral transmission of the Y net (Fig. 1) could partly explain why it had the

486   lowest growth in 2019, that negatively influenced fruit and shoot growths. Higher transmissions

487   of green and yellow light (Fig. 2B,C), along with lower ones of blue light portions (Fig. 2A)

488   were found, thus potentially explaining the lower tree development. Lower blue light

489   transmission has been proved to affect the development of foliar anatomy (Kong et al. 2012),

490   reducing the mesophyll layer (Pushnik et al. 1987; Saebo et al. 1995). The green light reversal

491   effect and inactivating properties of green-yellow photoreceptors may have further intensified

492   the lower performance of trees under the Y net compared to other treatments.

493   R net was mostly negative or intermediate, in terms of fruit weight and growth in both seasons

494   (Fig. 5B,D). This LE had the lowest amount of PAR and blue light, compared to the other LEs

495   (Fig. 2) and it is expectable that photosynthetic export may have been directed mainly to the

496   extension shoots, in fact, R shoots had accumulated more growth (Table 2) along with C ones.

497   Under the R net, probably the shade avoidance syndrome was triggered, caused by the lower

498   transmission of PAR and of also by blue light (Boini et al. 2020; Ballare et al. 1991; Pierik et

499 al. 2004; Keuskamp et al. 2011), which might have been translated in reduced mesophyll layer,

500 altering the leaf anatomy. As a result, photosynthetic performance could have been affected.

501 In 2019, B net appeared to negatively influence fruit growth (Fig. 5D), nevertheless, shoot

502 elongation resulted the highest (Table 2). The effect of pruning had a major influence on AER,

503 thus trees were not affected by the dwarfing effect of this LE. At harvest, however, B fruits

504 were among the smallest ones. It can be assumed that as for R, there was an imbalance of the

505 resources transfer.

506   It has to be pointed out that the heavy pruning before 2019's trial highly impacted on

507 the orchard's response. There is the possibility that nets shading at around 20% were not

508 effective enough to induce evident differences, or to surpass the intensity of the vegetative

509 outburst of the year 2019. In addition, several variables have not been considered, for example

510 the differences between the internal and external net temperature or the leaf gas exchange,

511 hence limiting the discussion of the results. Mupambi et al. (2018) explain why research that

512 involve light manipulation can give contradictory outcomes.

513

514   5. CONCLUSION

515   Photoselective nets are an important tool that can be used to modify the orchard

516 environment. Their application during the initial phase of fruit development can influence fruit

517 growth and shoot extension, impacting on the competition for carbohydrates.

518   Although differences occurring at harvest time may not reflect spring and early summer

519 growth (i.e. cell expansion stage was not considered), control and white nets appeared more

520 convenient, producing heavier fruit in both years. The blue and red nets did not give satisfactory

521 results in terms of final fruit weight, thus these nets are not suitable in the apple orchard during

522 cell division. This would apply to some apple producing areas in Italy, such as Southern Tyrol,

523   which possess environmental restrictions. Finally, yellow nets improved growth in the early

524   stages, however the spectral properties along the season did not help maintaining the same

525   trend, since final fruit weight was not among the highest.

526   Trees responses were heavily influenced by weather and orchard management, however,

527   the consistent results of final fruit weight, for both years, highlights how the light spectrum had

528   an impact on the development and growth of apple fruit.

529   Research should try even earlier than 20 DAFB, to manipulate light quality. It is

530   therefore necessary to approach this new technology, with further studies focusing on the

531   differences between each single factor that could improve and extend the use of photoselective

532   nets.

533

537

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